THE TAXONOMIC STATUS OF THE ECTOSYMBIOTIC FLATWORM *DIDYMORCHIS PARANEPHROPIS* HASWELL

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Didymorchis paranephropis Haswell, 1900 which inhabits the branchial chamber of freshwater crayfish from New Zealand is the type species of the genus: its taxonomic status with relation to the Temnocephalida has been uncertain. We show it to have a multisyncytial epidermis and thus to belong in the Temnocephalida. The pattern of the mosaic of epidermal syncytia in this species differs slightly from those found in Australian species assigned to the genus. The Australian and New Zealand species currently assigned to *Didymorchis* are clearly members of the Temnocephalida, and they belong rightfully in their own family Didymorchidae Bresslau & Reisinger, 1933. The Australian species show several differences from the type species, especially in the form of the sclerotised male copulatory organ. The biogeography of the Didymorchidae suggests a Gondwanan lineage that predates the isolation of New Zealand in the upper Cretaceous (ca. 80mya) consistent with the idea that they are a group derived early in the evolution of the Temnocephalida. □ *Temnocephalida, Didymorchidae, crayfish, ectosymbionts.*

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Didymorchis paranephropis Haswell, 1900 was described as a small ectosymbiotic worm which inhabits the branchial chamber of the New Zealand (NZ) parastacid crayfish *Paranephrops zealandicus* (White, 1847). Haswell (1900) considered it a rhabdocoel turbellarian in the family Dalyelliidae, though he remarked on its resemblance to temnocephalans. Two further species (*D. cherapsis* Haswell, 1915 and *D. astacopsidis* Haswell, 1915) were described from Australian crayfish hosts. Haswell (1915) noted some differences, for example, in the excretory system and sclerotised male copulatory organ, and assigned the Australian species to the genus with some reservation. *Didymorchis* has been considered either a dalyelliid (Haswell, 1915; von Graff, 1904-1908; Baer, 1953, 1961; Karling, 1957; Williams, 1981; Cannon, 1986; Rohde & Watson, 1990a, b) or a temnocephalan (see Bresslau & Reisinger, 1933; Fernando, 1934; Fyfe, 1942; Hyman, 1951; Schaefer, 1971; Joffe, 1981, 1988; Joffe et al., 1995a; Rohde, 1994; Watson & Rohde, 1995a, b). Bresslau & Reisinger (1933) even proposed its own family, Didymorchidae, which several authors have accepted.

Bresslau & Reisinger (1933) believed *Didymorchis* was transitional between Temnocephalida and 'Dalyellioida' while Fernando (1934) considered them intermediate between the 'primitive' Scutariellidae and the more 'advanced' Temnocephalidae. Based on the distribution of temnocephalans and of their hosts, Schaefer (1971) supported the temnocephalan affinity of the Didymorchidae. His view was that *Didymorchis* was a primitive temnocephalan from which the Temnocephalidae, Scutariellidae and Actinodactylellidae were derived. Joffe (1981) and Joffe et al. (1995a) suggested that *Didymorchis* was derived early in the evolution of the Temnocephalida.

Why has there been uncertainty about the position of *Didymorchis*? The worms normally glide on cilia just as do most rhabdocoels, but Haswell (1900) claimed they showed evidence of looping, a character Williams (1981) postulated characteristic of temnocephalans. Temnocephalans have anterior tentacles according to Williams (1981) and *Didymorchis* lacks tentacles. More significantly, the sclerotised male copulatory organ of the type species figured by Haswell (1900) is very like that of dalyelliids, whereas that of Australian and South American species assigned currently to the genus are like that of a typical temnocephalan cirrus (see Haswell, 1915; Baer, 1953, 1961; Karling, 1957; Joffe, 1981, 1982). Baer (1953, 1961), who placed *Didymorchis* in the Dalyelliidae, suggested the male copulatory organ of *D. paranephropis* is typical of simple dalyelliids. According to Karling (1957), however, it is closer to the strongly modified male copulatory organ of the dalyelliid *Hammalovortex nigrifrons* (Karling, 1934), though, as with

temnocephalans, it could be derived from that of *Alexlutheria*, the presumed primitive basal form of the dalyelliids. A South American species, *D. haswelli* Mañé-Garzon, 1960, and a variety, *D. haswelli* var. *australis* Dioni, 1972, have been described more recently with cirri close to those of Australian *Didymorchis* and thus typical of temnocephalans.

No new species of *Didymorchis* have been described since 1972, but ultrastructural studies of undetermined species of *Didymorchis* from Australian crayfish have provided support for the temnocephalan affinities of the Australian worms (see Rohde, 1987a, b; Rohde & Watson, 1990a, b; Watson & Rohde, 1995a, b). That the Australian species are temnocephalans was resolved by Joffe et al. (1995a) who determined that *D. cherapsis* and 3 undescribed species of *Didymorchis* from Australia had a 'multisyncytial epidermis', a characteristic of temnocephalans. The epidermis of temnocephalans is unique for the Platyhelminthes. It is syncytial (multinucleate fields arising through fusing of original cells) but comprised of several plates (Williams, 1986; Joffe et al., 1995a, b; Tyler & Tyler, 1997; Joffe & Cannon, 1998a). Some turbellarians have a syncytial epidermis but in the Neodermata, the whole body is, by contrast, covered by a single syncytium unbroken by cell membranes (Ehlers, 1985; Rieger et al., 1991; Joffe et al., 1995a; Tyler & Tyler, 1997; Joffe & Cannon, 1998a). *Udonella* is the only member of the Neodermata known with a separate syncytial field around its posterior sucker which is distinct from the remainder of its syncytial epidermis (Rohde & Watson, 1995; Tyler & Tyler, 1997). Joffe & Cannon (1998a) asserted that all described dalyelliids and dalyellioids have a cellular rather than syncytial epidermis. This is overwhelming evidence that the character 'syncytial epidermis composed of several syncytia' is a valid synapomorphy (a shared derived character) to distinguish the Temnocephalida from the family Dalyelliidae and/or suborder 'Dalyellioida'. Many of the characters used to historically discriminate temnocephalans from dalyelliids and other turbellarians are now known to be wrong or they are disputed.

The question of whether *Didymorchis* is temnocephalan nevertheless remains unanswered because the type species, *D. paranephropis*, from NZ has not been examined since Haswell (1900). Simply, does *D. paranephropis* from NZ have the syncytial mosaic typical of temnocephalans including the Australian species currently assigned to the genus, and concomittently is the Didymorchidae a temnocephalan taxon? We aimed to solve these questions by examination of the epidermal topography of *D. paranephropis* and an analysis of characters used historically to define this controversial taxon.

MATERIALS AND METHODS

Six specimens of the crayfish *Paranephrops zealandicus* were collected at Powder Ck., Otago, NZ (45°61'S; 170°25'E) in April 1995 by Tim Dodgsun (Department of Zoology, University of Otago, NZ), fixed in hot (ca. 60-90°C) 10% phosphate buffered formalin (HF) then transferred to 70% ethanol (etoh) and freighted to Brisbane. These provided fixed material of the worms. In March 1997, live worms were examined by LRGC in Dunedin from crayfish collected from the Taieri R., at 097867 on map 1-J44 'Dunedin' of the NZ Map Service in the second week of December 1996 by Ushio Nishitawa (Department of Zoology, University of Otago, NZ).

Live worms were observed using a stereo-microscope with transmitted and/or incident illumination. Worms were killed and fixed by flooding with a solution of 2% silver nitrate heated to ca. 60°C, washed in distilled water then exposed to bright sunlight or light from a cold light source for about 15-30 min. and either dehydrated down to 70% etoh and mounted in polyvinyl acetate (PVA) or dehydrated to 100% etoh and mounted in Euparal (Romeis, 1968; Joffe et al., 1995a, b).

Worms for wholemounts (WM) and scanning electron microscopy (SEM) were obtained from the host fixed in HF (see above). Hot fixation more clearly reveals the borders between the syncytia of the epidermal mosaic for SEM than does either ambient or cold fixation (Sewell & Cannon, 1995). Worms fixed for WM were rinsed in distilled water, stained with either Mayer's Haematoxylin (after Humason, 1972) (Hx), dehydrated in etoh, cleared in xylene and mounted in Canada balsam. Worms fixed for SEM, were rinsed 6 times in distilled water, dehydrated for 10 min in each of a graded series of ethanol, critical point dried, mounted on stubs and examined with a Hitachi S-530 SEM operating at 25kV. A Robinson backscatter detector was used to reduce charging. For examination of the sclerotic male copulatory organ, worms were placed in de Faure's medium (deF) either alive or after fixation in HF and rinsing in distilled water (Cannon & Sewell, 1995).

Photographs for figures were scanned from 35mm slides and negative film onto Kodak PhotoCD™, edited and assembled into plates using

Adobe Photoshop™ . Lineart illustrations for figures were prepared by pen and ink, scanned using a Hewlett Packard flatbed scanner, edited and assembled into figures using Adobe Illustrator™.

TERMINOLOGY AND MEASUREMENTS. Terminology follows the conventions established by Cannon & Sewell (1995) and updated by Sewell & Cannon (1998). Specimens are deposited in the QM and slides are designated as either wholemounts (WM), de Faure's cirrus preparations (CP) or silver nitrate preparation (SN). The registered material examined is listed in the order: QM registration number, specimen/slide preparation details followed by only those data which are different from that of the preceding registration. For clarity, discrete blocks of registrations are separated by semi-colons.

Plasticity of shape and further distortion caused by the effects of fixative indicate that measurements are valuable only as a guide to the approximate size and shape of the worms and their internal structures. All measurements were made with the aid of a drawing tube. A range followed usually by the mean in parentheses is provided for each measurement; estimates are qualified and are preceded by ca. Where no range is provided the measurements were the same for all worms. Measurements are in μm unless otherwise stated. Descriptions of epidermal topography follow the terminology established for Australian *Didymorchis* spp. (see Joffe et al., 1995a).

DIDYMORCHIDAE
Bresslau & Reisinger, 1933
Didymorchis Haswell, 1900

TYPE SPECIES. *Didymorchis paranephropis* Haswell, 1900.

OTHER SPECIES. *D. astacopsidis* Haswell, 1915. *D. cherapsis* Haswell, 1915.

SPECIES INQUIRENDAE. *D. haswelli* Mañé-Garzon, 1960. *D. haswelli* var. *australis* Dioni, 1972.

Didymorchis paranephropis Haswell, 1900
(Figs 1A, 2-3)

MATERIAL. QMGL18670-18672, GL18674 (WM) ex branchial chamber *Paranephrops zealandicus* from Powder Ck., Otago, NZ 45°61'S; 170°25'E April 1995 Dodgsun, T./ Sewell, K.B. HF/Hx; GL18673 (CP[3], 3 adult specimens (1 damaged)) HF/deF; GL18677 (SN[5]) from Taieri R., Otago, NZ March 1997 Nishitawa, U./Cannon, L.R.G.; GL18676 (CP[2], 2 adult specimens) deF: plus observation of living worms.

DECRIPTION. *D. paranephropis* was described by Haswell (1900) and is revised here. Small dorsoventrally compressed worms to ca. 1mm long and less than 300μm wide which inhabit the branchial chamber of their host and move by slow ciliary gliding. With posterior adhesive regions supplied with duct openings of rhabdite-forming glands. Pharynx cylinder-shaped and 'doliiform', ca $^1/_6$ th of the length of the body. Pigment absent except for that contained in a pair of eyes positioned posterior to pharynx. Body ciliated on the ventral surface but cilia absent from the lateral margins and dorsal surface. Excretory system with pores opening on the exterior on each side of ventral surface near the lateral margin. Male reproductive system composed of 2 somewhat oval-shaped testes situated close to the posterior border of the body; a copulatory bulb, sclerotised male copulatory organ with a tubular or rather funnel-like basal portion, and a distal portion composed of a number of spines; the basal portion is very wide at the proximal end, the greatest width being nearly equal to the length; spines are 10 or 12 in number; some straight and dagger-shaped, others curved and tapering. Female reproductive system with a single ovary, oviduct and uterus, paired vitelline glands, vitelline ducts and a vesicula resorbens but lacking a copulatory bursa.

The following measurements are provided to supplement Haswell's data. (Measurements based on 3 wholemount specimens QMGL18670-18671, GL18674 and 2 undamaged cirrus preparations GL18673).

External. Body from posterior margin to tip of anterior lobe 355-500 (413), to eyes 281-403 (334) long and 130-147 (136) wide.

General Anatomy. Pharynx 64-114 (89) long, 45-67 (57) wide. Eyes crescent-shaped ca 12 long, 5 wide and 33-42 (37) apart.

Reproductive System. Female. Ovary 20-28 (25) long, 16-19 (18) wide. Vesicula resorbens 23-33 (27) long, 17-27 (21) wide.

Male. Testes 73-106 (86) long, 28-48 (40) wide. Copulatory bulb 22-25 (23) long, 16-25 (20) wide. Male copulatory organ shaft cone-shaped, proximal opening 31-34 (33) wide, with narrow rim. Introvert (presumed) base angled, 21-23 (22) wide at base, lacks swelling and obvious distal opening. Spines large (all >15 long).

HOSTS. *Paranephrops zealandicus* (White, 1847): Parastacidae.

LOCALITY. Otago Region, NZ.

REMARKS. Type specimens were not located in the collections of either the Otago Museum, Dunedin, NZ, or the Australian Museum, Sydney, or

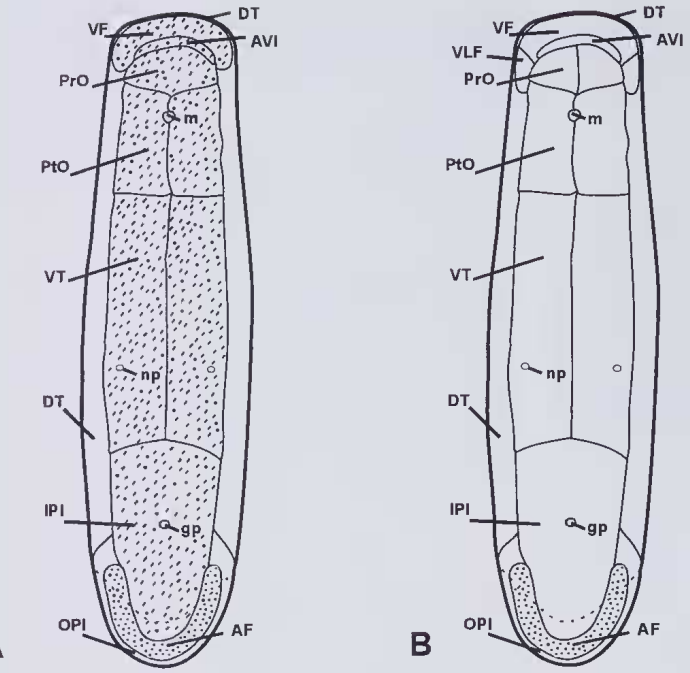

FIG. 1. A, mosaic of epidermal syncytia for *Didymorchis paranephropis* in ventral view. B, mosaic of epidermal syncytia suggested for the archetype of the Temnocephalida in ventral view. AF = adhesive field syncytium; AVI = anterior ventral intermediate syncytium; DT = dorsal trunk syncytium; gp = gonopore; IPI = inner posterior intermediate syncytium; m = mouth; np = nephridiopore; OPI = outer posterior intermediate syncytium; PrO = preoral syncytium; PtO = postoral syncytia; VLF = ventral lateral syncytia; VF = ventral frontal syncytium; VT = ventral trunk syncytia (terminology after Joffe et al., 1995a). Locomotory cilia are represented by fine oblique dashes.

the McLeay Museum, University of Sydney, Australia. Haswell, apparently failed to lodge type specimens. There is, however, no doubt that the worms redescribed here are specimens of *D. paranephropis* which remains the only species of the genus known from NZ.

Epidermis. Haswell (1900) noted that ciliation was confined to the ventral body and the epidermis is a 'thin protoplasmic layer with nuclei at wide intervals', but later (Haswell, 1915) he says of *Didymorchis* that the integument 'is entirely devoid both of cell-boundaries and of nuclei', inferring that both NZ and Australian representatives had a syncytial epidermis with insunk nuclei. Mañé-Garzon (1960) described and figured scarce, though well stained, intraepidermal nuclei of *Didymorchis haswelli* from South America, but also figured, without comment (see Mañé-Garzon, 1960 plate III, Fig. 1), structures which may be interpreted as cytoplasmic processes to insunk nuclei. Insunk nuclei are characteristic of Australian *Didymorchis* (see Rohde & Watson, 1990a; Joffe et al., 1995a). Intraepidermal nuclei, however, have also been demonstrated in an unidentified Australian *Didymorchis* by Rohde & Watson (1990a) who found such nuclei rarely and always isolated from the epidermis by a membrane (see Rohde & Watson, 1990a [Fig. 17]).

Silver nitrate staining and SEM both revealed an epidermal mosaic (Figs 1A, 3). The epidermis of *D. paranephropis* has 11 syncytia (Fig. 1A) of which 2 are 'paired': dorsal trunk syncytium (DT), ventral frontal syncytium (VF), anterior ventral intermediate syncytium (AVI), preoral syncytium (PrO), postoral syncytia (PtO) (paired), ventral trunk syncytia (VT) (paired), inner posterior intermediate syncytium (IPI), outer posterior intermediate syncytium (OPI) and adhesive field syncytium (AF). These can be divided into 6 'groups': ciliated syncytia over the ventral side of the body except the frontal part (2 VTs, 2 PtOs and PrO); the main unciliated epidermal syncytium (DT); syncytia of the ventral anterior part of the body (VF); syncytium of the adhesive field (AF); small syncytia that separate the adhesive field from the major epidermal syncytia (IPI & OPI); and the AVI. These 6 groups were found also in Australian species referred to *Didymorchis* by Joffe et al. (1995a) who related them to structure and function.

Comparison between the mosaic of *D. paranephropis* and the Australian species shows *D. paranephropis* has 11 syncytia of which 2 are 'paired' while the Australian species have 12-14 syncytia of which 2-3 are 'paired' [Joffe et al. (1995a) (Fig. 1A)]. There are other differences in the mosaic: 1, the VT of *D. paranephropis* is divided by a mid ventral suture, i.e. the VT is 'paired' sensu Joffe et al. (1995a), though the nephridiopores of all species are found in the VT; 2, no VLF are present in *D. paranephropis*; 3, the gonopore is located in the IPI of *D. paranephropis*, and within the VT of the Australian species; 4, the IPI of *D. paranephropis* is covered with dense locomotory cilia whereas ciliation is

FIG. 2. Cirrus of 2 adult specimens of *D. paranephropis* QMGL18673 ex type host and locality. Note that the cirrus spines of the specimen on the right are spread more widely than those of the specimen on the left and are articulated at their proximal insertion. Scale = 100μm.

sparse in the Australian species; and 5, the OPI circumscribes the posterior region of the body of *D. paranephropis* (Figs 1A, 3A-G) whereas in the Australian species, the entire OPI is on the ventral surface. Although only a small number of specimens was available for examination, intraspecific variability in the mosaic was observed; the junction of the paired VT and PtO was offset from the mid-line in several specimens (Fig. 3B).

Locomotion. Didymorchis paranephropis moves only by ciliary gliding. The worm becomes elongate, with the anterior end and the posterior adhesive field carried above the substrate when gliding in a straight line. The anterior end regularly 'searches' the substrate but was never observed to attach the worms firmly during locomotion. *D. paranephropis* alters course by gentle lateral movements of the body to achieve slight angle changes in direction. Large angle changes are accomplished by first attaching the posterior adhesive field, rotating the body about the attachment, releasing the attachment and then moving off in the new direction. *D. paranephropis* invariably contracts the posterior of the body forward before placing the adhesive region, which is well supplied with rhabdites, down onto the substrate; this is particularly noticeable when worms, gliding linearly with the body elongated, encounter an obstacle in their path. The pad may be observed to flatten and widen at the exact moment of contact with the substrate. Haswell (1900) claimed that *D. paranephropis* were able to 'loop' using attachment of both the anterior lobe and posterior adhesive pad , but we did not observe the worms to move in this way. Specimens in a thin film of water between a glass slide and coverslip, however, were seen to progress forwards using 'peristaltic' movements of the body and internal organs, an action which superficially resembles looping but which does not include attachment of the anterior lobe to the substrate. This form of locomotion is therefore an artifact and simply is a response to restricted movement such as that caused by the pressure of a coverslip.

Male Copulatory Organ. The distinctive form of the sclerotised male copulatory organ was figured by Haswell (1900) (compare Haswell (1900 [Plate XXI, 3]) with Fig. 2). Unlike the cirrus of *Didymorchis cherapsis*, *D. astacopsidis*, *D. haswelli* and *D. haswelli* var. *australis*, it lacks an apparent introvert with an obvious introvert base and introvert swelling, characters 'typical' of temnocephalans. Eversion of the male organ could not be determined in the fixed material, nor was it observed to evert when seen alive, though such an event is not readily induced and its possibility cannot be ruled out. The shaft and proximal articulations of the spines of the copulatory organ were enclosed in a robust band of muscle. Individual spines were observed to move/articulate, though only a few degrees.

Excretory System. Haswell (1900) claimed the excretory system of *D. paranephropis* opened on the ventral surface through two pores lateral to the posterior extremity of the pharynx, and that internal to the pores was a granular mass through which the duct coiled. Though clearly evident in even juveniles of small temnocephalans such as *Craspedella* spp., careful examination of live *D. paranephropis* and all the available wholemounts failed to reveal any evidence of excretory ampullae in the NZ worms, so it is unclear to what 'granular mass' Haswell was referring. Later, in describing the excretory system of Australian representatives of *Didymorchis*, Haswell (1915) noted the excretory pores lacked any 'intercalation of any excretory sac or vesicle'. He said also the pores in the Australian species were more posterior, though we observed the tiny nephridiopores of *D. paranephropis* are located, as in the Australian *Didymorchis*, about in the same relatively posterior position. Finally, Haswell (1915) said of the Australian species the arrangement of ducts 'differs completely from that which occurs in *D. paranephropis*', though

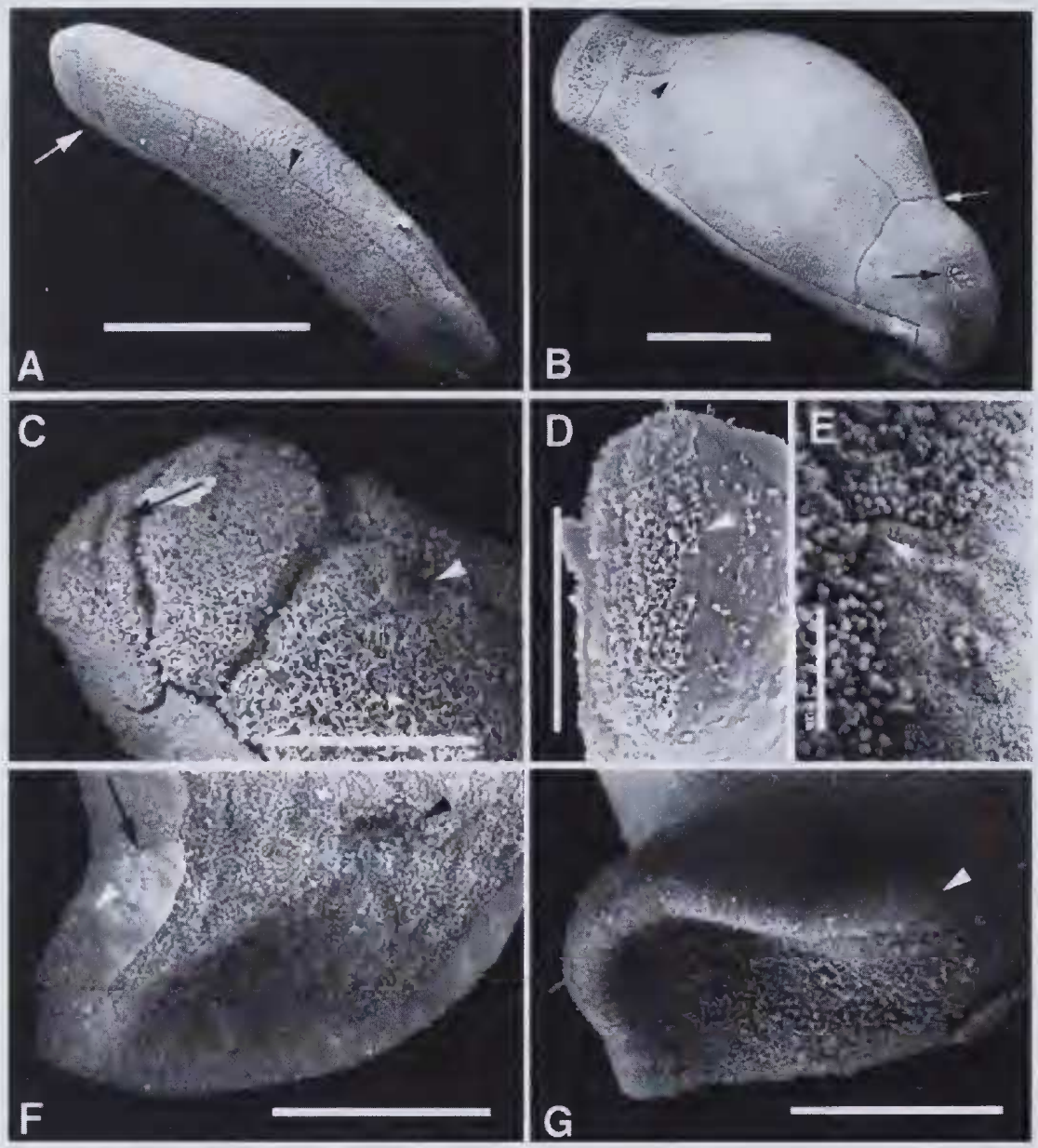

FIG. 3. SEM epidermal topography of *D. paranephropis* fixed in HF. Terminology for the syncytia from Joffe et al. (1995a) and Fig. 1. A, ventrolateral view of whole specimen showing pattern of the epidermal mosaic and position of the mouth (arrow). Arrowhead indicates border between the DT and the VT. Scale = 200μm. B, ventral view of whole specimen with posterior end curled dorsally at a point just posterior to the border between the OPI and DT (white arrowhead) to show the border between the VT and IPI (white arrow) and position of the gonopore (arrow). Note the 'offset' junction of the paired VT and PtO (black arrowhead) typical of most individuals examined. Scale = 100μm. C, ventrolateral view of anterior end showing dense locomotory cilia and pattern of the epidermal mosaic with position of the AVI indicated by an arrow and the mouth by an arrowhead. Scale = 50 μm. D, en face view of the anterior end showing the VF. The border of the VF and DT is indicated by an arrowhead. Scale = 50μm. E, lateral view of right side of the anterior end to show junction of the borders of the VF, AVI and DT (arrowhead). Scale = 10μm. F, ventral view of the poster end to show position of the gonopore (black arrowhead) and border between the OPI and DT (arrow) and border between the AF and the OPI (white arrowhead). Scale = 50μm. G, posterior end to show horse-shoe shaped AF and border between the AF and the OPI (arrowhead). Scale = 50μm.

his account seems insufficiently different to warrant such a strong statement.

DISCUSSION

The type species *D. paranephropis* is temnocephalan because it has a multisyncytial epidermis (Joffe & Cannon, 1998a). Furthermore, the similarity of the epidermal mosaic with that of the Australian representatives of the genus attests to their close relationship and confirms Didymorchidae as a recognisable taxon. Although it is not yet confirmed for all temnocephalan genera, a multisyncytial body wall epidermis has been described for all temnocephalans examined for the character (see Nichols, 1975, Williams, 1975, 1979, 1981, 1982, 1986, 1991, 1992; Joffe & Dzhanashvili, 1981; Joffe, 1982, 1988; Joffe et al., 1995a, b; Sewell & Cannon, 1995, in press; Sewell, 1998; Joffe & Cannon, 1998a). Joffe et al. (1995a) and Joffe & Cannon (1998a) suggested that the total number of syncytia has been reduced in the evolution of temnocephalans, i.e. the apomorphic (derived or advanced) state has fewer syncytia. *D. paranephropis* with 11 syncytia, shows more apomorphic characters than the 4 Australian species in the genus with 12-14 syncytia (Joffe et al., 1995a). The relative importance in the mosaic of 'paired' or isolated neighbouring syncytia with the same structure and 'groups' of syncytia correlated by their functional specialisations should, however, be considered in any attempt to elucidate the evolution of the epidermal mosaic (Joffe & Cannon, 1998a). In any case, the differences in the mosiac between the Australian and NZ species and the interspecific and intraspecific variation between Australian *Didymorchis* observed by Joffe et al. (1995a), support the hypothesis of Joffe & Cannon (1998a) that the ancestral form had more syncytia than are retained by the extant species. Joffe & Cannon (1998a) suggested that the mosaics of the Scutariellidae and the Didymorchidae arose independantly from a primitive stage characterised by a higher number of syncytia covering the trunk and a horseshoe-shaped AF syncytium. We propose an archetypal mosaic for the Temnocephalida (Fig. 1B) based on the total number of syncytia now recorded for species of *Didymorchis* (this study; Joffe et al., 1995a; Joffe & Cannon, 1998a) and on the model of evolution of the epidermal mosaic proposed by Joffe & Cannon (1998a).

Presumably, the unique multisyncytial epidermis of temnocephalans has evolved as an adaption to symbiosis in a freshwater environment, however, many questions remain to be resolved about the phylogeny of the epidermis, particularly between turbellarians and the Neodermata (Tyler & Tyler, 1997; Joffe & Cannon, 1998a). It is paradoxical that the primitive temnocephalan, *Didymorchis*, has insunk nuclei similar to those in the Neodermata, whereas other, presumably more advanced, temnocephalans all have intraepidermal nuclei, a character believed generally to be more primitive (Rohde, 1987a; Joffe et al., 1995a; Tyler & Tyler, 1997).

There is mounting support for the argument that temnocephalans are 'dalyellids' that have become ectosymbiotic and developed new characters (Ehlers, 1985; Ehlers & Sopott-Ehlers, 1993; Rohde et al., 1993, 1995; Rohde, 1996; Joffe & Cannon, 1998b). The characters used historically to separate *D. paranephropis* from temnocephalids are either wrong or at best debatable. Baer (1931) and Williams (1981) maintained a looping method of locomotion was the main characteristic that separated temnocephalans from other turbellarians. Furthermore, Williams (1986) stated that temnocephalans lacked locomotory cilia and had anterior tentacles. These characters are wrong, because *D. paranephropis*, like the undisputed temnocephalan *Dicerato-cephala boschmai* Baer, 1953, do not loop and move only by locomotory cilia (Cannon, 1991; Jones & Lester, 1992; Joffe et al., 1995b). Fyfe (1942) was correct when she asserted that the absence of tentacles in *Didymorchis* was not sufficient reason to exclude it from the Temnocephalida.

The sclerotised male copulatory organ of *D. paranephropis* differs from the cirrus figured for *D. cherapsis* and *D. astacopsidis* from Australia (Haswell, 1915, figs 6,7) and *D. haswelli* (see Mañé-Garzon, 1960, pl. 4, figs 1,2) and *D. haswelli* var *australis* (see Dioni, 1972, figs 7-11) from South America. The cirrus in the Australian and South American species is 'typical' of most temnocephalans with an introvert lined with rows of relatively short spines. By contrast, the male copulatory organ of *D. paranephropis* has only a few extremely large and elongate spines which appear to be articulated individually by muscles and may not form a 'typical' introvert. Moreover, a large band of muscle surrounds the base and proximal spine articulation, and the organ apparently lacks an 'introvert swelling' (sensu Cannon & Sewell, 1995). The differences between the size and number of spines in the Australian and NZ species were noted by Haswell (1915). The arrangement of these large spines in *D. paraneph-*

ropis certainly resembles the 'typical' male intromittent organ of the Dalyelliidae (Baer, 1953; Luther, 1955), however, recent studies on the Craspedellinae by Cannon & Sewell (1995) and Sewell & Cannon (1998) confirm that the shape and form of the temnocephalan cirrus is not a reliable character to discriminate genera. *Craspedella pedum* Cannon & Sewell, 1995 for example has a unique cirrus with a strongly-reflexed shaft and a permanently everted introvert composed of flat, plate-like teeth, whereas all other species of the Craspedellinae (in the genera *Craspedella*, *Heptacraspedella*, *Zygopella* and *Gelasinella*) have, by contrast, a typical temnocephalan cirrus (Cannon & Sewell, 1995; Sewell & Cannon, 1998). Thus, although the form of the cirrus in the Australian species assigned to *Didymorchis* is more typically temnocephalan than that of the type species of the genus, the differences in our opinion, considering the other similarities between the worms, do not warrant erection of a new genus for the Australian species.

The presence of an epidermal mosaic has not been confirmed for the South American species assigned to *Didymorchis* and thus their status as temnocephalans is not yet established firmly. The morphology of the reproductive structures in *D. haswelli* and *D. haswelli* var. *australis* from South America, particularly that of the cirrus, nevertheless suggests that they too are temnocephalan and probably closer to the Australian than to the NZ species. Nevertheless, until the temnocephalan status of the South American species is confirmed, the temnocephalan family Didymorchidae originally erected by Bresslau & Reisinger (1933) can accommodate with certainty only the Australian and NZ species assigned currently to *Didymorchis*

We propose therefore the following family diagnosis:

DIDYMORCHIDAE
Bresslau & Reisinger, 1933

Temnocephalida with elongate cylindrical body, without lateral ridges and lacking tentacles. Epidermis composed of 11-14 epidermal syncytia. Pigment confined to a single pair of anterodorsal eyes. Posterior adhesive field (attachment organ) not pedunculate or circular and without a marginal valve. Dorsal surface without imbricating scales or raised body ridges. Locomotory cilia confined to the ventral surface. Buccal cavity or pre-pharynx conspicuous; pharynx large and directed anteroventrally. Gut without colour or septa. Eyes present, small, discrete and widely spaced. Gonopore midventral in posterior third of body. Seminal receptacle single; vitellaria restricted to a pair of lateral cylinders. Testes, 1 pair posterior, spherical to ovoid, positioned posterior to gut. Small, slender worms ca 1mm in vivo. Inhabiting the branchial chamber of their freshwater crustacean hosts.

Didymorchidae occur on parastacid crayfish in Australia and NZ and have been described from South America, therefore, they have an 'amphinotic' or 'circumantarctic' Gondwanan lineage (Banarescu, 1990). The ancient Gondwanan distribution of *Didymorchis* is consistent with the hypothesis that the Didymorchidae are an early derived group of Temnocephalida . In Australia the Didymorchidae are found on both *Cherax* and *Euastacus* crayfish and they have a widespread distribution. *Didymorchis* were described by Haswell (1915) from crayfish in eastern Australia and he mentioned an 'allied form' from a Tasmanian crayfish *Astacopsis australiensis* Haswell, 1882 [according to Clark (1936) this crayfish species is a secondary synonym of *Euastacus serratus* but as *Euastacus* does not occur in Tasmania (Hamr, 1992), the identity of this host remains uncertain]. The worms also occur on *Cherax* from Western Australia and the Northern Territory (unpublished observations). The absence of the Didymorchidae from India and Africa and their presence in NZ, suggests that they evolved after India, but before NZ separated from Gondwanaland (Heatwole, 1987). Alternately, the worms may never have existed in the African and Indian regions of Gondwanaland or they may have become extinct in these regions after the breakup of the southern supercontinent. We predict that the Australian and South American species of *Didymorchis* will be shown to differ less from each other than from the type species, *D. paranephropis,* because of the earlier separation of NZ from Gondwanaland.

Finally, the controversy about the taxonomic position of the ectosymbiont *Varsoviella kozminskii* Gieysztor & Wiszniewski, 1947 from the gills of gammarids from the Vistula R. in Poland is analogous to that for *D. paranephropis* before our study. Like *D. paranephropis*, *V. kozminskii* has been classified hesitantly as a dalyelliid, with attention drawn to its temnocephalan similarities (Gieysztor & Wiszniewski, 1947; Luther, 1955; Matjasic, 1990; Ehlers & Sopott Ehlers, 1993; Rohde, 1996). The sclerotised male copulatory organ of *V. kozminskii* is strikingly similar in general form to that of *D. paranephropis*. Like *D. paranephropis*, the male organ of *V. kozminskii*

has a few large dagger-shaped spines, and *V. kozminskii* has not been examined since it was first described. It may, therefore, be instructive to examine the epidermis of *V. kozminskii* to help resolve its taxonomic position. Matjasic (1990) proposed that, if a temnocephalan, *Varsoviella* could be placed in its own family Varsoviellidae.

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